PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PLOUGMANN, VINGTOFT & PARTNERS
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen
DANEMARK

Date of mailing (day/month/year)
23 April 1998 (23.04.98)

Applicant's or agent's file reference
P199600322 WO

IMPORTANT NOTIFICATION

International application No.
PCT/DK97/00342

International filing date (day/month/year)
25 August 1997 (25.08.97)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

HOFMAN-BANG & BOUTARD, LEHMANN & REE A/S
Hans Bekkevolds Allé 7
DK-2900 Hellerup
Denmark

State of Nationality:

State of Residence:

Telephone No.: 45 39 48 80 00

Facsimile No.: 45 39 48 80 80

Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

PLOUGMANN, VINGTOFT & PARTNERS
Sankt Annæ Plads 11
P.O. Box 3007
DK-1021 Copenhagen
Denmark

State of Nationality:

State of Residence:

Telephone No.: +45 33 63 93 00

Facsimile No.: +45 33 63 96 00

Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Beate Giffo-Schmitt

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00342

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 15/11 // C12N 15/63
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, MEDLINE, EMBASE, BIOSIS, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9425609 A1 (HYBRITECH INCORPORATED), 10 November 1994 (10.11.94) --	1-57
X	Nucleic Acids Research, Volume 16, No 15, 1988, Arnold R. Oliphant et al, "Defining the consensus sequences of E.coli promoter elements by random selection" page 7673 - page 7683 --	1-57
A	Dialog Information Services, file 155, MEDLINE, Dialog accession no. 08012585, Medline accession no. 94368841, Nilsson D et al: "A conserved sequence in tRNA and rRNA promoters of Lactococcus latis"; & Biochim Biophys Acta Sep 13 1994, 1219 (1) p141-4 --	16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 1998	04 -02- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00342

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Services, file 155, MEDLINE, Dialog accession no. 07764569, Medline accession no. 94172317, Eaton T et al: "Cloning and sequence analysis of the dnak gene region of Lactococcus lactis subsp. lactis"; & J Gen Microbiol Dec 1993, 139 (Pt 12) p3253-64 --	17
A	Dialog Information Services, file 155, MEDLINE, Dialog accession no. 08484065, Medline accession no. 96076630, Casas C et al: "Sequence analysis of a 9873 bp fragment of the left arm of yeast chromosome XV that contains the ARG8 and CDC33 genes, a putative riboflavin synthase beta chain gene, and four new open reading frames"; & Yeast Sep 15 1995, 11 (11) p1061-7 -- --------	26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ DK 97/00342

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-57

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

07/01/98

International application No.
PCT/DK 97/00342

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9425609 A1	10/11/94	AU 6776194 A	21/11/94

PCT/DK97/00342

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENTS

(PCT Administrative Instructions, Section 411)

To:

HOFMAN-BANG & BOUTARD, LEHMANN & REE A/S
Hans Bekkevolds Allé 7
DK-2900 Hellerup
DANEMARK

Date of mailing (day/month/year)
06 October 1997 (06.10.97)

Applicant's or agent's file reference
P199600322 WO

IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/DK97/00342	25 August 1997 (25.08.97)	23 August 1996 (23.08.96)

Applicant
JENSEN, Peter, Ruhdal et al

The applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to the following application(s).

Priority application No:	Priority date:	Priority country:	Date of receipt of priority document:
0886/96	23 Aug 1996 (23.08.96)	DK	19 Sep 1997 (19.09.97)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Asseaff

Telephone No.: (41-22) 338.83.38

WO 98/07846
PCT/DK97/00342

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

HOFMAN-BANG & BOUTARD, LEHMANN & REE A/S
Hans Bekkevolds Allé 7
DK-2900 Hellerup
DANEMARK

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 26 February 1998 (26.02.98)	
Applicant's or agent's file reference P199600322 WO	**IMPORTANT NOTICE**
International application No. PCT/DK97/00342	**International filing date** (day/month/year) 25 August 1997 (25.08.97)
Priority date (day/month/year) 23 August 1996 (23.08.96)	
Applicant JENSEN, Peter, Ruhdal et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

   AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,SK,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AL,AM,AP,AT,AZ,BA,BB,BG,BY,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,HU,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NZ,OA,PT,RO,RU,SD,SE,SG,SI,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZW

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on

   26 February 1998 (26.02.98) under No. WO 98/07846

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year)	IMPORTANT NOTICE
26 February 1998 (26.02.98)	
Applicant's or agent's file reference	**International application No.**
P199600322 WO	PCT/DK97/00342

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

09/242657 19

PATENT COOPERATION TREATY

REC'D 15 JAN 1999
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
20911 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/11		
Applicant		
JENSEN, Peter Ruhdal		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

corrected version

Date of submission of the demand	Date of completion of this report
18/03/1998	10. 12. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Claes, B Telephone No. (+49-89) 2399-8429

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK97/00342

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-69	as originally filed			
10a	as received on	28/11/1998	with letter of	26/11/1998

Claims, No.:

1-22	as received on	28/11/1998	with letter of	26/11/1998

Drawings, sheets:

1/5-5/5	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK97/00342

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,9,11-21
	No:	Claims	1-3,5-8,10,22
Inventive step (IS)	Yes:	Claims	9,11,14-17
	No:	Claims	1-8,10,12,13,18-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Note: The amendments to the present application are allowable under Article 34(2)(b) PCT.

1. The following documents are referred to:

   D1 = Nilsson & Johansen (1994), Biochemica et Biophysica, 1219, p.141-144.
   D2 = Oliphant & Struhl (1988), NAR, 16(15), 7673-7683.

2.1. D1 discloses (see Fig.2 at p.143) a promoter library (i.e. a collection of promoters) comprising a set of different individual promoter sequences covering a range of promoter strengths, the sense strand of these promoters comprising at least two consensus sequences (see legend) which are kept constant and between said consensus sequences or flanking at least one, a nucleotide spacer sequence at least part of which is varied to comprise nucleotides that are selected randomly among A, T, C or G see e.g. between -35 and -15 or preceding -44).

2.2. Claim 1 of the amended application requires the promoter library to be "suitable for optimising the expression of a gene in a selected organism or group of organisms". It is at present not fully clear what this qualification should technically mean. However, the library as disclosed in D1 must also be considered suitable for optimising the expression of a gene in a selected organism, as this organism may e.g. be considered any organism. Furthermore, the IPEA considers that the different indicated promoters very unlikely all have the same activity. Hence, they have a range of activities. Certainly this library is suitable for optimising (e.g. see which promoter is the strongest) expression of "said gene" e.g. in L.lactis, L.cremoris or Bacillus.

   Claim 1 also contains the amendment that th promoter library is "**spanning, with respect to promoter activities for said gene, a range of interest, in small steps, each step preferably changing the ativity by 50-100%**" (emphasis added). It is clear from the wording "preferably" that the last part of the sentense

is optional. Hence the feature merely reads "**spanning, with respect to promoter activities for said gene, a range of interest, in small steps**". In view of the fact that "small steps" is a relative expression which in the present case appears not to constitute any technical limitation in the absense of the concrete indication to the size of the steps. In fact, as the different promoters of the D+ library all have different activities, this library must also be considered to span a range of promoter activities "in small steps".

Applicant contends that the description of the present application allegedly makes claer to the skilled person that when there is referred to "random selection of nucleotides" the intention is clearly to indicate that a distribution of the individial nucleotides in the library is attempted that does not substatially deviate from a 1:1:1:1 distribution.
The IPEA cannot follow this reasoning. No such suggestion can be found in the description. Furthermore, it is of the opinion that the wording "is varied to comprise nucleotides that are selected randomly among the nucleobases A,T,C and G" merely suggests that for each of the indicated position either an A, T, C **or** is present. It is certainly not understood that obligatorily all four bases need to be present. Hence, the above referred to wording is not considered to give any indication of the size of the library, i.e. that for each position all four possibilities should be present.

In view of the above assessment the subject matter of present claims 1-3,5-8 and 10 lacks novelty under Article 33(2) PCT.

Note: In view of the novelty objections raised above, the argumentation of the applicant in support of inventive step of the claimed subject matter becomes irrelevant as it relies on features in the claims which are not considered to provide novelty and can therefore also not constitute essential technical features suitable to base an inventive step on.

The subject matter of claim 4 can in the light of modern design of gene technology tools not be considered to involve inventive activity (Article 33(3) PCT).

2.3. No proper document seems to be cited in the International Search Report which

would qualify for prejudicing the novelty of the subject matter of claims 12 and 13. However, any listing in the literature, before the relevant date for the present application, of eukaryotic promoter sequences with variations between the TATA box and a UAS would be destroy the novelty of the subject matter of the said claims.

In absence of such a document, though, claims 12 and 13 are objected to under for lack of inventive step as the skilled person would without inventive skill list and compare the sequences of available eukaryotic promoter sequences (e.g. to identify consensus sequences and arrive at a similar list as is present in Fig.2 of D1. Hence, the subject matter of claims 12 and 13 is not acceptable under Article 33(3) PCT.

2.4. Furthermore, the identification method of the e.g. the strongest promoter of the list of promoters (either prokaryotic or eukaryotic) in a certain organism cannot be accepted to involve an inventive step. Hence, claims 18-21 contravenes Article 33(3) PCT.

3. Claims 9,11,14 and 15 further define the promoter library of claim 1 to contain a or certain physical characteristic (technical definition of the library) or by certain specific sequences it needs to contain.
Claim 16 defines a method for constructing a set of promoters for optimising the expression of a gene based on varying the spaces between two consensus sequences in a given set of known promoters.

D2 is considered to represent the closest prior art for the subject matter of claim 16. D2 discloses the exchange of one of the consensus sequences in a set of promoters in order to optimise the expression of the promoters. Nowhere in the document there is an indication that variation of the spacer sequence between or flanking the consensus sequences could result in an optimised activity. Neither could such an incentive be found in any of the other documents cited in the ISR. Hence, the subject matter of claims 16 and 17 is acknowledged to be new and to involve an inventive step (Article 33(2)(3) PCT). In view of the above assessment the subject matter of claims 9,11,14 and 15 likewise can be accepted to be novel and to involve an inventive step (Article 33(2)(3) PCT).

4. The subject matter of claim 22 is actually directed to any promoter which is active (theoretically the library from which it is selected could be any arbitrary library). Hence, the subject matter of claim 22 lacks novelty under Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The wording "essentially" in claim 1 and furthermore e.g. in claim 16 for defining the constant feature of part of the consensus sequence introduces unclarity in the scope of protection for the claims on file. Either a part is constant or not constant. Unclear is however how constant "essentially constant" is (Article 6 PCT objection).

2. An analogous objection as above is valid for the wording "and minor variations thereof" as applied in claim 11 (Article 6 PCT objection).

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09/242657

REC'D 15 DEC 1998

WIPO PCT

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
20911 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/11		
Applicant		
JENSEN, Peter Ruhdal		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/03/1998	10.12.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Claes, B Telephone No. (+49-89) 2399-8429

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK97/00342

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-69	as originally filed			
10a	as received on	28/11/1998	with letter of	26/11/1998

Claims, No.:

1-22	as received on	28/11/1998	with letter of	26/11/1998

Drawings, sheets:

1/5-5/5	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,9,11-21
	No:	Claims	1-3,5-8,10,22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Note: The amendments to the present application are allowable under Article 34(2)(b) PCT.

1. The following documents are referred to:

   D1 = Nilsson & Johansen (1994), Biochemica et Biophysica, 1219, p.141-144.
   D2 = Oliphant & Struhl (1988), NAR, 16(15), 7673-7683.

2.1. D1 discloses (see Fig.2 at p.143) a promoter library (i.e. a collection of promoters) comprising a set of different individual promoter sequences covering a range of promoter strengths, the sense strand of these promoters comprising at least two consensus sequences (see legend) which are kept constant and between said consensus sequences or flanking at least one, a nucleotide spacer sequence at least part of which is varied to comprise nucleotides that are selected randomly among A, T, C or G see e.g. between -35 and -15 or preceding -44).

2.2. Claim 1 of the amended application requires the promoter library to be "suitable for optimising the expression of a gene in a selected organism or group of organisms". It is at present not fully clear what this qualification should technically mean. However, the library as disclosed in D1 must also be considered suitable for optimising the expression of a gene in a selected organism, as this organism may e.g. be considered any organism. Furthermore, the IPEA considers that the different indicated promoters very unlikely all have the same activity. Hence, they have a range of activities. Certainly this library is suitable for optimising (e.g. see which promoter is the strongest) expression of "said gene" e.g. in L.lactis, L.cremoris or Bacillus.

   Claim 1 also contains the amendment that th promoter library is "**spanning, with respect to promoter activities for said gene, a range of interest, in small steps, each step preferably changing the ativity by 50-100%**" (emphasis added). It is clear from the wording "preferably" that the last part of the sentense

is optional. Hence the feature merely reads "**spanning, with respect to promoter activities for said gene, a range of interest, in small steps**". In view of the fact that "small steps" is a relative expression which in the present case appears not to constitute any technical limitation in the absense of the concrete indication to the size of the steps. In fact, as the different promoters of the D+ library all have different activities, this library must also be considered to span a range of promoter activities "in small steps".

Applicant contends that the description of the present application allegedly makes claer to the skilled person that when there is referred to "random selection of nucleotides" the intention is clearly to indicate that a distribution of the individial nucleotides in the library is attempted that does not substatially deviate from a 1:1:1:1 distribution.
The IPEA cannot follow this reasoning. No such suggestion can be found in the description. Furthermore, it is of the opinion that the wording "is varied to comprise nucleotides that are selected randomly among the nucleobases A,T,C and G" merely suggests that for each of the indicated position either an A, T, C **or** is present. It is certainly not understood that obligatorily all four bases need to be present. Hence, the above referred to wording is not considered to give any indication of the size of the library, i.e. that for each position all four possibilities should be present.

In view of the above assessment the subject matter of present claims 1-3,5-8 and 10 lacks novelty under Article 33(2) PCT.

Note: In view of the novelty objections raised above, the argumentation of the applicant in support of inventive step of the claimed subject matter becomes irrelevant as it relies on features in the claims which are not considered to provide novelty and can therefore also not constitute essential technical features suitable to base an inventive step on.

The subject matter of claim 4 can in the light of modern design of gene technology tools not be considered to involve inventive activity (Article 33(3) PCT).

2.3. No proper document seems to be cited in the International Search Report which

would qualify for prejudicing the novelty of the subject matter of claims 12 and 13. However, any listing in the literature, before the relevant date for the present application, of eukaryotic promoter sequences with variations between the TATA box and a UAS would be destroy the novelty of the subject matter of the said claims.

In absence of such a document, though, claims 12 and 13 are objected to under for lack of inventive step as the skilled person would without inventive skill list and compare the sequences of available eukaryotic promoter sequences (e.g. to identify consensus sequences and arrive at a similar list as is present in Fig.2 of D1. Hence, the subject matter of claims 12 and 13 is not acceptable under Article 33(3) PCT.

2.4. Furthermore, the identification method of the e.g. the strongest promoter of the list of promoters (either prokaryotic or eukaryotic) in a certain organism cannot be accepted to involve an inventive step. Hence, claims 18-21 contravenes Article 33(3) PCT.

3. Claims 9,11,14 and 15 further define the promoter library of claim 1 to contain a or certain physical characteristic (technical definition of the library) or by certain specific sequences it needs to contain.
Claim 16 defines a method for constructing a set of promoters for optimising the expression of a gene based on varying the spaces between two consensus sequences in a given set of known promoters.

D2 is considered to represent the closest prior art for the subject matter of claim 16. D2 discloses the exchange of one of the consensus sequences in a set of promoters in order to optimise the expression of the promoters. Nowhere in the document there is an indication that variation of the spacer sequence between or flanking the consensus sequences could result in an optimised activity. Neither could such an incentive be found in any of the other documents cited in the ISR. Hence, the subject matter of claims 16 and 17 is acknowledged to be new and to involve an inventive step (Article 33(2)(3) PCT). In view of the above assessment the subject matter of claims 9,11,14 and 15 likewise can be accepted to be novel and to involve an inventive step (Article 33(2)(3) PCT).

4. The subject matter of claim 22 is actually directed to any promoter which is active (theoretically the library from which it is selected could be any arbitrary library). Hence, the subject matter of claim 22 lacks novelty under Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The wording "essentially" in claim 1 and furthermore e.g. in claim 16 for defining the constant feature of part of the consensus sequence introduces unclarity in the scope of protection for the claims on file. Either a part is constant or not constant. Unclear is however how constant "essentially constant" is (Article 6 PCT objection).

2. An analogous objection as above is valid for the wording "and minor variations thereof" as applied in claim 11 (Article 6 PCT objection).

In other aspects, the invention pertains to a method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the above method of constructing a promoter library, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation,

and a promoter sequence that is capable of optimising the expression of a gene in a selected organism, which promoter sequence is obtainable by the above method of isolating a promoter sequence.

International patent application No. PCT/DK97/00342
Peter Rudahl Jensen
Artificial promoter libraries for selected organisms and promoters derived from such libraries
Our ref: 20911 PC 1

NEW CLAIMS, 26 NOVEMBER 1998

1. A promoter library suitable for optimising the expression of a gene in a selected organism or group of organisms, said library comprising a set of different individual promoter sequences covering, with respect to promoter strength for said gene, a range of promoter activities, the set of different individual promoter sequences comprising double stranded DNA sequences, the sense strands of which comprise

at least two consensus sequences of a promoter sequence identified in said organism or group of organisms, at least half of each of said consensus sequences is kept constant in all of the individual promoter sequences and, between said consensus sequences or flanking at least one of said consensus sequences, a nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among the nucleobases A, T, C and G,

the promoter library spanning, with respect to promoter activitities for said gene, a range of interest, in small steps, each step preferably changing the activity by 50-100%.

2. A promoter library according to claim 1 wherein at least 10 nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

3. A promoter library according to claim 1 wherein the promoter sequences comprise a regulatory DNA sequence imparting a specific regulatory feature to the promoters of said promoter sequences.

AMENDED SHEET

4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease

5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.

6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -10 signal TATAAT.

7. A promoter library according to claim 5 or 6 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -35 signal TTGACA.

8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.

9. A promoter library according to any of claims 5-8 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42

10. A promoter library according to claim 7 wherein the spacer sequence between the -35 and the -10 signal is 14-23 bp.

11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.

12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.

13. A promoter library according to claim 12 wherein the consensus sequences comprise a TATA box and at least one upstream activation sequence (UAS).

14. A promoter library according to claim 12 or 13 wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

15. A promoter library according to any of claims 12-14 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method comprising

(i) selecting from the promoter library of any of claims 1-15 a set of promoters covering a desired range of promoter activities,

AMENDED SHEET

(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and a eukaryotic organism.

21. A method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16 or 17, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation.

22. A promoter sequence that is capable of optimising the expression of a gene in a selected organism, the promoter sequence is obtainable by the method of claim 21.

PATENT COOPERATION TREATY

09/242657

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PLOUGMANN VINGTOFT & PARTNERS

23 NOV 1998

PCT

WRITTEN OPINION

(PCT Rule 66)

To:

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Fax 45 33 63 96 00

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Date of mailing *(day/month/year)* 19.11.98

Applicant's or agent's file reference
20911 PC 1

REPLY DUE within [redacted] **7 days** from the above date of mailing

International application no.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996

International Patent Classification (IPC) or both national classification and IPC
C12N15/11

Applicant
JENSEN, Peter Ruhdal

1. This written opinion is the ~~first~~ second drawn up by this International Preliminary Examining Authority.
2. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and / or arguments, see Rule 66.4bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 23/12/1998

Name and mailing address of the international preliminary examining authority

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Form PCT/IPEA/408 (Cover sheet) (January 1994)

WRITTEN OPINION

International application No. PCT/DK97/00342

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-69 as originally filed

Claims, No.:

1-21 as received on 26/08/1998 with letter of 26/08/1998

Drawings, sheets:

1/5-5/5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-3,5-8,10,21
Inventive step (IS)	Claims	4,12,13,18-20
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents are referred to:

D1 = Nilsson & Johansen (1994), Biochemica et Biophysica, 1219, p.141-144.
D2 = Oliphant & Struhl (1988), NAR, 16(15), 7673-7683.

2.1. D1 discloses (see Fig.2 at p.143) a promoter library (i.e. a collection of promoters) comprising a set of different individual promoter sequences covering a range of promoter strengths, the sense strand of these promoters comprising at least two consensus sequences (see legend) which are kept constant and between said consensus sequences or flanking at least one, a nucleotide spacer sequence at least part of which is varied to comprise nucleotides that are selected randomly among A, T, C or G see e.g. between -35 and -15 or preceding -44).

2.2. Claim 1 of the amended application requires the promoter library to be "suitable for optimising the expression of a gene in a selected organism or group of organisms". It is at present not fully clear what this qualification should technically mean. However, the library as disclosed in D1 must also be considered suitable for optimising the expression of a gene in a selected organism, as this organism may e.g. be considered any organism. Certainly this library is suitable for optimising (e.g. see which promoter is the strongest) expression e.g. in L.lactis, L.cremoris or Bacillus.

In view of the above assessment the subject matter of present claims 1-3,5-8 and 10 (Article 33(2) PCT).

The subject matter of claim 4 can in the light of modern design of gene technology tools not be considered to involve inventive activity (Article 33(3) PCT).

2.3. No proper document seems to be cited in the International Search Report which would qualify for prejudicing the novelty of the subject matter of claims 12 and 13.

However, any listing in the literature, before the relevant date for the present application, of eukaryotic promoter sequences with variations between the TATA box and a UAS would be destroy the novelty of the subject matter of the said claims.

In absence of such a document, though, claims 12 and 13 are objected to under for lack of inventive step as the skilled person would without inventive skill list and compare the sequences of available eukaryotic promoter sequences (e.g. to identify consensus sequences and arrive at a similar list as is present in Fig.2 of D1. Hence, the subject matter of claims 12 and 13 is not acceptable under Article 33(3) PCT.

2.4. Furthermore, the identification method of the e.g. the strongest promoter of the list of promoters (either prokaryotic or eukaryotic) in a certain organism cannot be accepted to involve an inventive step. Hence, claims 18-20 contravenes Article 33(3) PCT.

3. Claims 9,11,14 and 15 further define the promoter library of claim 1 to contain a or certain physical characteristic (technical definition of the library) or by certain specific sequences it needs to contain.
Claim 16 defines a method for constructing a set of promoters for optimising the expression of a gene based on varying the spaces between two consensus sequences in a given set of known promoters.

D2 is considered to represent the closest prior art for the subject matter of claim 16. D2 discloses the exchange of one of the consensus sequences in a set of promoters in order to optimise the expression of the promoters. Nowhere in the document there is an indication that variation of the spacer sequence between or flanking the consensus sequences could result in an optimised activity. Neither could such an incentive be found in any of the other documents cited in the ISR. Hence, the subject matter of claims 16 and 17 is acknowledged to be new and to involve an inventive step (Article 33(2)(3) PCT). In view of the above assessment the subject matter of claims 9,11,14 and 15 likewise can be accepted to be novel and to involve an inventive step (Article 33(2)(3) PCT).

4. The subject matter of claim 21 is actually directed to any promoter which is active (theoretically the library from which it is selected could be any arbitrary library). Hence, the subject matter of claim 21 lacks novelty under Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The wording "essentially" in claim 1 and furthermore e.g. in claim 16 for defining the constant feature of part of the consensus sequence introduces unclarity in the scope of protection for the claims on file. Either a part is constant or not constant. Unclear is however how constant "essentially constant" is (Article 6 PCT objection).

2. An analogous objection as above is valid for the wording "and minor variations thereof" as applied in claim 11 (Article 6 PCT objection).

Concluding remarks:

The attention of the Applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed, Article 34(2)(b) PCT.

When filing amended claims the applicant should at the same time bring the description into conformity with the amended claims. Care should be taken during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Article 34(2)(b) PCT).

In order to facilitate the examination of the conformity of the amended application with the requirements of Article 34(2)(b) PCT, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion (if the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed), **and to indicate the passages of the application as filed on which these amendments are based.**

It is noted in this context that the present written opinion does not contain an opinion on the allowability of the amended claims under Article 34(2)(b) PCT. As it would appear, at present, the claims are allowable. However, the Applicant is invited to indicate where in the application as originally filed there is formal support for the wordings as now applied in the claims.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PLOUGMANN; VINGTOFT & PARTNERS
Sankt Annae Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

PCT

NOTIFICATION CONCERNING INFORMAL COMMUNICATIONS WITH THE APPLICANT

(PCT Rule 66.6)

	Date of mailing *(day/month/year)* 19.11.98
Applicant's or agent's file reference 20911 PC 1	**TRANSMITTAL FOR INFORMATION**
International application no. PCT/DK97/00342	International filing date *(day/month/year)* 25/08/1997
Applicant JENSEN, Peter Ruhdal	

An informal communication took place on , between the International Preliminary Examining Authority and the applicant / the agent.

A copy of the note on that communication (Form PCT/IPEA/428) is herewith transmitted for your information.

Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer DW20244 Telephone No. (+49-89) 2399-

Form PCT/IPEA/429 (july 1993)

Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens
Patent Cooperation Treaty
Traîté de coopération en matière de brevets

PCT

Application No.: PCT/DK97/00342

Note on an informal communication by telephone with the Applicant

A copy of this note is being sent to the Applicant for information

To Support Service: return the file to 1st examiner !

Participants

Applicant: Jensen et al.

Agent: Henry Sogaard

Examiner(s): Claes, B

Summary of the communication

1. The examiner and the Representative agreed on sending a second written opinion basied on the newly filed claims. The time limit set for answering to this second written opinion was agreed on to be 7 days.

18/11/1998
.....
Date *(day / month / year)*

.....
Claes, B
Authorized officer of IPEA

09/242657

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BY TELEFAX AND CONFIRMATION BY MAIL

26 August 1998

International Patent Application No. PCT/DK97/00342
Publication No. WO 98/07846
Peter Ruhdal Jensen
Artificial promoter libraries for selected organisms...
Our ref: 20911 PC 1

Dear Sirs,

In your first written opinion you have stated that the claims as originally drafted cannot form the basis for an assessment of novelty and inventive step.

The Applicant has taken due note of this statement and has, in consequence hereof, redrafted the claims so as to make it clear what falls under the scope of the claims.

In new claim 1, it is now an essential feature that the consensus sequences in all members of the promoter library are kept constant, whereas sequences between or flanking the consensus sequences are varied randomly.

This feature is also included in the independent claims 16 and 18.

Previous claims 27-29 relating to a promoter as such have been deleted. New independent claim 21 relates to a promoter sequence, which is selected from a set of promoters constructed using the method of claim 16 as the sequence of the set of promoters that in a given organism shows optimised flux of formation of a given gene product.

Applicant trusts that the new claims can form a suitable basis for an assessment of novelty and inventive step.

With respect to novelty, applicant submits that none of the prior art cited in the international search report discloses a promoter library comprising a set of

promoters having essentially constant consensus sequences and randomly varied "spacer" sequences.

Also, there is no suggestion in the prior art that it is possible to optimise gene expression by selecting a promoter that, relative to a naturally occurring promoter regions, is modified in a sequence between or flanking the consensus sequences. Accordingly, the invention provides a significant contribution to the art, which is entirely original.

In case the IPEA cannot fully acknowledge novelty and inventive step for the invention as presently claimed, the Applicant would be grateful to receive a further written opinion or, if possible remaining issued are minor, to have the opportunity to discuss such issues with the Examiner over the telephone.

Yours sincerely,

Plougmann, Vingtoft & Partners

Henry Søgaard

New set of claims, in triplicate
Form 1037

International patent application No. PCT/DK97/00342
Peter Rudahl Jensen
Artificial promoter libraries for selected organisms and promoters derived from such libraries
Our ref: 20911 PC 1

NEW CLAIMS, AUGUST 1998

1. A promoter library suitable for optimising the expression of a gene in a selected organism or group of organisms, said library comprising a set of different individual promoter sequences covering, with respect to promoter strength, a range of promoter activities, the set of different individual promoter sequences comprising double stranded DNA sequences, the sense strands of which comprise

at least two consensus sequences of a promoter sequence identified in said organism or group of organisms, at least half of each of said consensus sequences is kept essentially constant in all of the individual promoter sequences and, between said consensus sequences or flanking at least one of said consensus sequences, a nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among the nucleobases A, T, C and G,

2. A promoter library according to claim 1 wherein at least 10 nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

3. A promoter library according to claim 1 wherein the promoter sequences comprise a regulatory DNA sequence imparting a specific regulatory feature to the promoters of said promoter sequences.

4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease

5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.

6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the –10 signal TATAAT.

7. A promoter library according to claim 5 or 6 wherein the consensus sequences comprise at least 3 conserved nucleotides of the –35 signal TTGACA.

8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.

9. A promoter library according to any of claims 5-8 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42

10. A promoter library according to claim 7 wherein the spacer sequence between the –35 and the –10 signal is 14-23 bp.

11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.

12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.

13. A promoter library according to claim 12 wherein the consensus sequences comprise a TATA box and at least one upstream activation sequence (UAS).

14. A promoter library according to claim 12 or 13 wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

15. A promoter library according to any of claims 12-14 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences essentially constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method comprising

(i) selecting from the promoter library of any of claims 1-15 a set of promoters covering a desired range of promoter activities,

(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and an eukaryotic organism.

21. A promoter sequence that is capable of optimising the expression of a gene in a selected organism, the promoter sequence is obtained by constructing, using the method of claim 16, a set of promoters which is suitable for optimising the expression of the gene in said organism and selecting, among the set of promoter sequences, the sequence that in the selected organism shows optimised flux of formation of the product of the gene.

PLOUGMANN, VINGTOFT & PARTNERS

09/242657

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BY TELEFAX AND CONFIRMATION BY MAIL

26 November 1998

International Patent Application No. PCT/DK97/00342
Publication No. WO 98/07846
Peter Ruhdal Jensen
Artificial promoter libraries for selected organisms...
Our ref: 20911 PC 1

Dear Sirs,

In response to your second written opinion, dated 19 November 1998, the Applicant submits a new set of claims and a new page 10a of the description where the subject matter of new claims 21-22 is briefly stated. In addition, the below comments and arguments are submitted.

The Applicant wants to express his appreciation for your giving him the opportunity to further consider your objections and to file amendments with a view to bringing the application into conformity with Article 33 PCT.

In the new set of claims, claim 1 is amended to incorporate the technical feature: "the promoter library spanning, with respect to promoter activities for said gene, a range of interest, in small steps, each step preferably changing the activity by 50-100%. For the sake of clarity only, the expression "for said gene" is inserted into the claim in line 3 of the claim.

The basis for this amendment is i.a. found on page 5, lines 13-15.

D1 discloses, as pointed out by you, in Fig. 2 a set of different individual promoters comprising at least two consensus sequences which are kept constant and a nucleotide spacer sequence at least part of which is varied. However, in your written opinion you appear to read two assumptions into the cited document which, as the Applicant reads D1, cannot be derived from that document. First, you are assuming

that the individual promoter sequences that are listed in Fig. 2 are covering a range of promoter strength and second, you are assuming that the spacer sequences are varied between the sequences by comprising nucleotides that are selected randomly among A, T, C or G.

In respect of the first assumption, the Applicant fails to recognise that D1 explicitly mentions such a feature, although it cannot be ruled out that the promoter strengths might be different. In respect of the second assumption, the Applicant wants to make the comment that, when there is referred to random selection of nucleotides in the specification, the intention is clearly to indicate that a distribution of the individual nucleotides in the library is attempted that does not substantially deviate from a 1:1:1:1 distribution such as it is described e.g. in D2.

Notwithstanding the above arguments, the Applicant has amended the claims by introducing the above feature into claim 1 and it is submitted that nowhere in the cited prior art is there any reference to a promoter library as now defined which spans an activity range of interest in small steps, such as a library where each step of activity change is in the range of 50-100%. Accordingly, the subject matter of the new claim 1 is novel over D1.

Furthermore, a new claim 21 is introduced, the subject-matter of which is a method of isolating a promoter sequence being capable of optimising the expression of a gene in a selected organism, the method comprising constructing a promoter library according to the method of claim 16 or 17 and selecting from that library as inserted into the selected organism, the clone hereof that shows optimised flux of gene product formation and isolating the promoter sequence from that clone. Previous claim 21, now claim 22 is amended by referring to the method of new claim 21.

It is submitted that the subject matter of new claim 21 does not extend beyond the content of the application as filed, see e.g. page 8, lines 28-35.

The technical problem with which the present invention is concerned may be seen as that of achieving metabolic flux optimisation by fine tuning expression of enzymes involved in a metabolic pathway rather than by simply achieving many fold overexpression of a single enzyme. As it is explained in the description, simply achieving overexpression of a gene as it is normally attempted when it is desired to optimise the output of a particular metabolite, does frequently not lead to the desired result (see e.g. page 2). Accordingly, the present inventors used a completely different approach, i.e. to use a promoter library covering the entire range of activities as a basis for selecting a promoter of the right strength to achieve the optimum metabolic flux of a given metabolite.

To obtain this is not simply a matter of selecting the strongest possible promoter, but rather a matter of identifying a promoter having a strength that results in the metabolic flux optimisation. Such a promoter may have any strength and, as it will be clear from the description, the specific promoter strength that for a given

metabolic flux is the optimum strength cannot be predicted but must be determined as it is described in the present specification. Therefore, for such an approach to succeed, it is required to construct a promoter library covering, in respect of promoter activity, the range which is desired for a particular gene and select the promoter that results in an optimum output of the metabolite. This in turn requires that a set of promoters is available where the change in activity from one individual promoter to another is in small steps.

As it is explained on page 2, modulation of the strength of promoters has been achieved prior to the effective date for the present application by basepair changes in the consensus sequences or by changes in the length of the spacer between them. However, as it is also explained, the impact of such changes on the promoter strength will be large, and accordingly, these known approaches were not feasible for achieving small steps of strength modulation.

The crux of the present invention is the highly unexpected finding that, contrary to what, prior to the effective date, was the general assumption in the art, modifications of the non-consensus sequences of promoter regions in both prokaryotic and eukaryotic promoter regions modulate the activity of the promoter. This finding made it possible to construct a promoter library as claimed permitting a hitherto not possible fine tuning of gene expression and metabolic flux optimisation.

It is therefore submitted that the subject matter of all the claims involves an inventive step.

In respect of your observations under Item VIII, the wording "essentially" in claims 1 and 16 has been deleted.

It is finally submitted that the Applicant has made a *bona fide* attempt to respond seriously and appropriately to your objections

For your convenience, we enclose a copy of the claims in which we have shown the amendments now performed as compared to the claims filed with our letter of 26 August.1998 (the above amendments are indicated (i) in bold (insertions) and (ii) by striking through (deletions), respectively).

Yours sincerely,

Plougmann, Vingtoft & Partners

Henry Søgaard

New claims, 26 November 1998
Copy of new claims with amendments indicated
New page 10a
Form 1037

26 NOV. 1998

International patent application No. PCT/DK97/00342
Peter Rudahl Jensen
Artificial promoter libraries for selected organisms and promoters derived from such libraries
Our ref: 20911 PC 1

NEW CLAIMS, 26 NOVEMBER 1998

1. A promoter library suitable for optimising the expression of a gene in a selected organism or group of organisms, said library comprising a set of different individual promoter sequences covering, with respect to promoter strength for said gene, a range of promoter activities, the set of different individual promoter sequences comprising double stranded DNA sequences, the sense strands of which comprise

at least two consensus sequences of a promoter sequence identified in said organism or group of organisms, at least half of each of said consensus sequences is kept constant in all of the individual promoter sequences and, between said consensus sequences or flanking at least one of said consensus sequences, a nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among the nucleobases A, T, C and G,

the promoter library spanning, with respect to promoter activitities for said gene, a range of interest, in small steps, each step preferably changing the activity by 50-100%.

2. A promoter library according to claim 1 wherein at least 10 nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

3. A promoter library according to claim 1 wherein the promoter sequences comprise a regulatory DNA sequence imparting a specific regulatory feature to the promoters of said promoter sequences.

4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease

5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.

6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -10 signal TATAAT.

7. A promoter library according to claim 5 or 6 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -35 signal TTGACA.

8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.

9. A promoter library according to any of claims 5-8 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42

10. A promoter library according to claim 7 wherein the spacer sequence between the -35 and the -10 signal is 14-23 bp.

11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.

12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.

13. A promoter library according to claim 12 wherein the consensus sequences comprise a TATA box and at least one upstream activation sequence (UAS).

14. A promoter library according to claim 12 or 13 wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

15. A promoter library according to any of claims 12-14 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method comprising

(i) selecting from the promoter library of any of claims 1-15 a set of promoters covering a desired range of promoter activities,

(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and a eukaryotic organism.

21. A method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16 or 17, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation.

22. A promoter sequence that is capable of optimising the expression of a gene in a selected organism, the promoter sequence is obtainable by the method of claim 21.

International patent application No. PCT/DK97/00342
Peter Rudahl Jensen
Artificial promoter libraries for selected organisms and promoters derived from such libraries
Our ref: 20911 PC 1

COPY OF NEW CLAIMS, 26 NOVEMBER 1998

with amendments shown as compared to the set of claims as filed on 26 August 1998 (bold = insertions; strike-through = deletions)

1. A promoter library suitable for optimising the expression of a gene in a selected organism or group of organisms, said library comprising a set of different individual promoter sequences covering, with respect to promoter strength **for said gene**, a range of promoter activities, the set of different individual promoter sequences comprising double stranded DNA sequences, the sense strands of which comprise

at least two consensus sequences of a promoter sequence identified in said organism or group of organisms, at least half of each of said consensus sequences is kept ~~essentially~~ constant in all of the individual promoter sequences and, between said consensus sequences or flanking at least one of said consensus sequences, a nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among the nucleobases A, T, C and G,

the promoter library spanning, with respect to promoter activitities for said gene, a range of interest, in small steps, each step preferably changing the activity by 50-100%.

2. A promoter library according to claim 1 wherein at least 10 nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

3. A promoter library according to claim 1 wherein the promoter sequences comprise a regulatory DNA sequence imparting a specific regulatory feature to the promoters of said promoter sequences.

4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease

5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.

6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the −10 signal TATAAT.

7. A promoter library according to claim 5 or 6 wherein the consensus sequences comprise at least 3 conserved nucleotides of the −35 signal TTGACA.

8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.

9. A promoter library according to any of claims 5-8 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42

10. A promoter library according to claim 7 wherein the spacer sequence between the −35 and the −10 signal is 14-23 bp.

11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.

12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.

13. A promoter library according to claim 12 wherein the consensus sequences comprise a TATA box and at least one upstream activation sequence (UAS).

14. A promoter library according to claim 12 or 13 wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

15. A promoter library according to any of claims 12-14 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences ~~essentially~~ constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method comprising

(i) selecting from the promoter library of any of claims 1-15 a set of promoters covering a desired range of promoter activities,

(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and a eukaryotic organism.

21. A method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16 or 17, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation.

22. A promoter sequence that is capable of optimising the expression of a gene in a selected organism, the promoter sequence is obtainable **by the method of claim 21.** ~~by constructing, using the method of claim 16, a set of promoters which is suitable for optimising the expression of the gene in said organism and selecting, among the set of promoter sequences, the sequence that in the selected organism shows optimised flux of formation of the product of the gene.~~

In other aspects, the invention pertains to a method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the above method of constructing a promoter library, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation,

and a promoter sequence that is capable of optimising the expression of a gene in a selected organism, which promoter sequence is obtainable by the above method of isolating a promoter sequence.

PATENT COOPERATION TREATY

09/242657

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PLOUGMANN VINGTOFT & PARTNERS

1 JULI 1998

PCT

HEP/BH

WRITTEN OPINION

(PCT Rule 66)

To:

PLOUGMANN; VINGTOFT & PARTNERS
Sankt Annae Plads 11
P.O. Box 3007
DK-1021 Copenhagen K
DANEMARK

Date of mailing *(day/month/year)*	29.06.98
Applicant's or agent's file reference: 20911 PC 1	**REPLY DUE** within **2 month(s)** from the above date of mailing

International application no.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996

International Patent Classification (IPC) or both national classification and IPC
C12N15/11

Applicant
JENSEN, Peter Ruhdal

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☐	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and / or arguments, see Rule 66.4bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 23/12/1998

Name and mailing address of the international preliminary examining authority European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer / Examiner Claes, B Formalities officer (incl. extension of time limits) Vullo, C Telephone No. (+49-89) 2399-8061

Form PCT/IPEA/408 (Cover sheet) (January 1994)

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

2. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☒ the entire international application,

☐ claims Nos. ,

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. all are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

   see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Ad item III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability.

1. Claims 1 of the present application defines its subject matter in such a way that for a skilled person it is impossible to judge whether a possible "promoter library" he has made falls under the scope of claim 1 or not. The following open questions render the scope of claim 1 completely obscure.

   * a) How many "artificial promoters" are contained in a "library"? Are it more than one promoter (i.e. more than 2) or does it need to be all possible variants defined?
   * b) What are previously known promoter sequences" and "promoter sequences isolated from natural source"? How can such promoters be excluded from a library? How can one know that a library does not contain such a promoter?
   * c) What exactly is meant by "a consensus sequence" in the context of claim 1 and furthermore what is "a part thereof comprising at least half of each". It appears that "consensus sequence" is an arbitrary definition which is open to interpretation.

   The application as originally filed does not give the answer to any of these questions.

   The definition of the "library" in claims 2-10,15,18-23 and 25 do not clarify the above questions. Hence, these claims are unclear.

2. Claims 11-14,24 answers in part the above question c). However, in view of the other unanswered questions the scope of the claim is too vague to give a meaningful assessment of novelty and inventive step.

3 Claims 16,17 and 26 in a first aspect make an attempt to define the "sequence" of the promoter library (i.e. by referring to SEQ ID No. 1-3). However this definition does still not solve some of the open questions referred to above. Furthermore, by the adding the wording "with minor variations in the consensus sequence sequences and spacer lengths" the scope of these claims is again such that the skilled person would impossibly be able to determine whether he works within the scope of these claims or not.

4. Claim 26 refers to any promoter contained in a library as defined in claim 1. Claim 28 to any promoter"derived" from the promoter in claim 26. In view of the above objections again the scope of claim 27 is obscure. Does it mean that any "not-known or non-naturally occurring promoter" is embraced by the claim? This type of "omnibus" claiming is not acceptable to be clear.

5. Claims 30-55 consist of method claims applying the same language as claims 1-25. The same objections as to claims 1-25 recited above apply to these claims.

6. As claims 56 and 57 rely on te definition of subject matter according to claims 1-26 and 30-55 again no meaningful opinion can be given for the subject matter of these claims.

Ad item IV. Lack of unity of invention.

The subject matter of claim 28 appears to lack unity of invention.

Promoter sequences comprising "consensus sequences" are considered to be known in the art. The common feature of the promoters defined in claim 28 is that they comprise "consensus sequences". Hence, each of the listed promoters consists of an independent solution to the problem of providing "additional or alternative" promoter sequences containing "consensus sequences". These promoters thus lack unity of invention, a posteriori.

PATENT COOPERATION TREATY

09/242657

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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DANEMARK

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PLOUGMANN VINGTOFT & PARTNERS
15 JAN. 1999
HEP/BH

PCT

Fax + Mail

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 15.01.99

Applicant's or agent's file reference
20911 PC 1

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996

Applicant
JENSEN, Peter Ruhdal

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Dear Sir,
Referring to your fax of 14.1.99, I send you the corrected sheet 2 of the IPER dated 10.12.98

Name and mailing address of the IPEA/
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Fax: (+49-89) 2399-4465

Authorized officer
Vullo, C
Tel. (+49-89) 2399-8061

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK97/00342

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,9,11-21
	No:	Claims	1-3,5-8,10,22
Inventive step (IS)	Yes:	Claims	9,11,14-17
	No:	Claims	1-8,10,12,13,18-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

PLOUGMANN, VINGTOFT & PARTNERS

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International Preliminary Examining
Authority
European Patent Office
Erhardtstrasse 27
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14 JAN. 1999

BY TELEFAX AND CONFIRMATION BY MAIL

14 January 1999

International Patent Application No. PCT/DK97/00342
Publication No. WO 98/07846
Peter Ruhdal Jensen
Artificial promoter libraries for selected organisms...
Our ref: 20911 PC 1

Dear Sirs,

On receiving the Preliminary Examination Report, we noted that the text on sheet 2 regarding "Box V", where it is stated that inventive step is not acknowledged for any of claims 1-22 is not consistent with the text of the section "re item V", sheet 3, where it is stated on item 3 that at least claims 9, 11 and 14-17 are accepted to be novel and to involve an inventive step.

The Applicant kindly requests to receive a corrected IPER and that you forward such a correction to the relevant elected offices.

Yours sincerely,

Plougmann, Vingtoft & Partners

Henry Søgaard

BH

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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DANEMARK

PLOUGMANN VINGTOFT & PARTNERS
14 DEC. 1998
HEP/BH

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	10. 12. 98
Applicant's or agent's file reference 20911 PC 1	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996

Applicant
JENSEN, Peter Ruhdal

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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14 DEC. 1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
20911 PC 1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK97/00342	25/08/1997	23/08/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/11		
Applicant		
JENSEN, Peter Ruhdal		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/03/1998	10. 12. 98
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Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK97/00342

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-69	as originally filed			
10a	as received on	28/11/1998	with letter of	26/11/1998

Claims, No.:

1-22	as received on	28/11/1998	with letter of	26/11/1998

Drawings, sheets:

1/5-5/5	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,9,11-21
	No:	Claims	1-3,5-8,10,22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Note: The amendments to the present application are allowable under Article 34(2)(b) PCT.

1. The following documents are referred to:

   D1 = Nilsson & Johansen (1994), Biochemica et Biophysica, 1219, p.141-144.
   D2 = Oliphant & Struhl (1988), NAR, 16(15), 7673-7683.

2.1. D1 discloses (see Fig.2 at p.143) a promoter library (i.e. a collection of promoters) comprising a set of different individual promoter sequences covering a range of promoter strengths, the sense strand of these promoters comprising at least two consensus sequences (see legend) which are kept constant and between said consensus sequences or flanking at least one, a nucleotide spacer sequence at least part of which is varied to comprise nucleotides that are selected randomly among A, T, C or G see e.g. between -35 and -15 or preceding -44).

2.2. Claim 1 of the amended application requires the promoter library to be "suitable for optimising the expression of a gene in a selected organism or group of organisms". It is at present not fully clear what this qualification should technically mean. However, the library as disclosed in D1 must also be considered suitable for optimising the expression of a gene in a selected organism, as this organism may e.g. be considered any organism. Furthermore, the IPEA considers that the different indicated promoters very unlikely all have the same activity. Hence, they have a range of activities. Certainly this library is suitable for optimising (e.g. see which promoter is the strongest) expression of "said gene" e.g. in L.lactis, L.cremoris or Bacillus.

   Claim 1 also contains the amendment that th promoter library is "**spanning, with respect to promoter activities for said gene, a range of interest, in small steps, each step preferably changing the ativity by 50-100%**" (emphasis added). It is clear from the wording "preferably" that the last part of the sentense

is optional. Hence the feature merely reads "**spanning, with respect to promoter activities for said gene, a range of interest, in small steps**". In view of the fact that "small steps" is a relative expression which in the present case appears not to constitute any technical limitation in the absense of the concrete indication to the size of the steps. In fact, as the different promoters of the D+ library all have different activities, this library must also be considered to span a range of promoter activities "in small steps".

Applicant contends that the description of the present application allegedly makes claer to the skilled person that when there is referred to "random selection of nucleotides" the intention is clearly to indicate that a distribution of the individial nucleotides in the library is attempted that does not substatially deviate from a 1:1:1:1 distribution.
The IPEA cannot follow this reasoning. No such suggestion can be found in the description. Furthermore, it is of the opinion that the wording "is varied to comprise nucleotides that are selected randomly among the nucleobases A,T,C and G" merely suggests that for each of the indicated position either an A, T, C **or** is present. It is certainly not understood that obligatorily all four bases need to be present. Hence, the above referred to wording is not considered to give any indication of the size of the library, i.e. that for each position all four possibilities should be present.

In view of the above assessment the subject matter of present claims 1-3,5-8 and 10 lacks novelty under Article 33(2) PCT.

Note: In view of the novelty objections raised above, the argumentation of the applicant in support of inventive step of the claimed subject matter becomes irrelevant as it relies on features in the claims which are not considered to provide novelty and can therefore also not constitute essential technical features suitable to base an inventive step on.

The subject matter of claim 4 can in the light of modern design of gene technology tools not be considered to involve inventive activity (Article 33(3) PCT).

2.3. No proper document seems to be cited in the International Search Report which

would qualify for prejudicing the novelty of the subject matter of claims 12 and 13. However, any listing in the literature, before the relevant date for the present application, of eukaryotic promoter sequences with variations between the TATA box and a UAS would be destroy the novelty of the subject matter of the said claims.

In absence of such a document, though, claims 12 and 13 are objected to under for lack of inventive step as the skilled person would without inventive skill list and compare the sequences of available eukaryotic promoter sequences (e.g. to identify consensus sequences and arrive at a similar list as is present in Fig.2 of D1. Hence, the subject matter of claims 12 and 13 is not acceptable under Article 33(3) PCT.

2.4. Furthermore, the identification method of the e.g. the strongest promoter of the list of promoters (either prokaryotic or eukaryotic) in a certain organism cannot be accepted to involve an inventive step. Hence, claims 18-21 contravenes Article 33(3) PCT.

3. Claims 9,11,14 and 15 further define the promoter library of claim 1 to contain a or certain physical characteristic (technical definition of the library) or by certain specific sequences it needs to contain.
Claim 16 defines a method for constructing a set of promoters for optimising the expression of a gene based on varying the spaces between two consensus sequences in a given set of known promoters.

D2 is considered to represent the closest prior art for the subject matter of claim 16. D2 discloses the exchange of one of the consensus sequences in a set of promoters in order to optimise the expression of the promoters. Nowhere in the document there is an indication that variation of the spacer sequence between or flanking the consensus sequences could result in an optimised activity. Neither could such an incentive be found in any of the other documents cited in the ISR. Hence, the subject matter of claims 16 and 17 is acknowledged to be new and to involve an inventive step (Article 33(2)(3) PCT). In view of the above assessment the subject matter of claims 9,11,14 and 15 likewise can be accepted to be novel and to involve an inventive step (Article 33(2)(3) PCT).

4. The subject matter of claim 22 is actually directed to any promoter which is active (theoretically the library from which it is selected could be any arbitrary library). Hence, the subject matter of claim 22 lacks novelty under Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The wording "essentially" in claim 1 and furthermore e.g. in claim 16 for defining the constant feature of part of the consensus sequence introduces unclarity in the scope of protection for the claims on file. Either a part is constant or not constant. Unclear is however how constant "essentially constant" is (Article 6 PCT objection).

2. An analogous objection as above is valid for the wording "and minor variations thereof" as applied in claim 11 (Article 6 PCT objection).

In other aspects, the invention pertains to a method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the above method of constructing a promoter library, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation,

and a promoter sequence that is capable of optimising the expression of a gene in a selected organism, which promoter sequence is obtainable by the above method of isolating a promoter sequence.

International patent application No. PCT/DK97/00342
Peter Rudahl Jensen
Artificial promoter libraries for selected organisms and promoters derived from such libraries
Our ref: 20911 PC 1

NEW CLAIMS, 26 NOVEMBER 1998

1. A promoter library suitable for optimising the expression of a gene in a selected organism or group of organisms, said library comprising a set of different individual promoter sequences covering, with respect to promoter strength for said gene, a range of promoter activities, the set of different individual promoter sequences comprising double stranded DNA sequences, the sense strands of which comprise

at least two consensus sequences of a promoter sequence identified in said organism or group of organisms, at least half of each of said consensus sequences is kept constant in all of the individual promoter sequences and, between said consensus sequences or flanking at least one of said consensus sequences, a nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among the nucleobases A, T, C and G,

the promoter library spanning, with respect to promoter activitities for said gene, a range of interest, in small steps, each step preferably changing the activity by 50-100%.

2. A promoter library according to claim 1 wherein at least 10 nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

3. A promoter library according to claim 1 wherein the promoter sequences comprise a regulatory DNA sequence imparting a specific regulatory feature to the promoters of said promoter sequences.

AMENDED SHEET

4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease

5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.

6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -10 signal TATAAT.

7. A promoter library according to claim 5 or 6 wherein the consensus sequences comprise at least 3 conserved nucleotides of the -35 signal TTGACA.

8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.

9. A promoter library according to any of claims 5-8 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42

10. A promoter library according to claim 7 wherein the spacer sequence between the -35 and the -10 signal is 14-23 bp.

11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.

12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.

13. A promoter library according to claim 12 wherein the consensus sequences comprise a TATA box and at least one upstream activation sequence (UAS).

14. A promoter library according to claim 12 or 13 wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

15. A promoter library according to any of claims 12-14 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method comprising

(i) selecting from the promoter library of any of claims 1-15 a set of promoters covering a desired range of promoter activities,

(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and a eukaryotic organism.

21. A method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16 or 17, a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation.

22. A promoter sequence that is capable of optimising the expression of a gene in a selected organism, the promoter sequence is obtainable by the method of claim 21.